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SHORT COMMUNICATION

EVIDENCE FOR ENZYMATIC THIOLTRANSACETYLATION IN PLANT EXTRACTS

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Key Word Index—*Phaseolus mungo*; Leguminosae; thiol-acetyl-transferase; thiol esters; sulfur metabolism.

Abstract—Triton X100 extracts prepared from acetone powders of mung bean roots acetylate β-mercaptoethanol and L-cysteine in the presence of acetyl–CoA. This activity can be destroyed by boiling the extracts. No activity could be detected if extracts were prepared without Triton X100, indicating that the enzyme or enzymes involved are probably membrane-bound.

INTRODUCTION

DURING experiments with extracts of mung bean roots designed to study the synthesis of acetylcholine catalyzed by choline acetyltransferase in the presence of acetyl–CoA and choline, we observed that sulfhydryl compounds which were present in the reaction mixture became acetylated. Enzymes (thiol acetyltransferases) which catalyze the transfer of the acetyl moiety of acetyl–CoA to sulfhydryl-containing acceptors have been demonstrated[1,2] to occur in animal tissues and bacteria, but their occurrence does not appear to be reported in plant tissues.

TABLE 1. ACETYLATION OF SULFHYDRYL COMPOUNDS BY EXTRACTS OF MUNG BEAN ROOTS

Reaction conditions	Net counts/min/0·4 ml reaction mixture	cpm/g fr. wt
Boiled extract + β-mercaptoethanol	64	—
Extract + β-mercaptoethanol	2550	22 900
Boiled extract + L-cysteine	79	—
Extract + L-cysteine	505	4550

* See experimental for reaction mixture.
† The data are the averages of 2 different experiments.

We studied transacetylation by Triton X100 extracts prepared from acetone powders of mung bean roots, which were initially designed to study choline acetyltransferase, using β-mercaptoethanol and L-cysteine as substrates. These two compounds have previously been shown to be substrates of animal and bacteria thiol acetyltransferases.[1] The assay employed is similar to that developed by Schrier and Shuster[3] to determine choline acetyltransferase and based on the use of [1-^{14}C]acetyl–CoA followed by separation on an anion exchange resin of the thiol ester formed from the radioactive acetyl–CoA. Table 1 shows

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[1] R. O. BRADY and E. R. STADTMAN, *J. Biol. Chem.* **211**, 621 (1954).
[2] I. C. GUNSALUS, L. S. BARTON and W. GRUBER, *J. Am. Chem. Soc.* **78**, 1763 (1956).
[3] B. K. SCHRIER and L. SHUSTER, *J. Neurochem.* **14**, 977 (1967).

that the extracts used acetylate β-mercaptoethanol and L-cysteine and this activity can be almost completely destroyed by boiling. The slight radioactivity observed in the effluents of the boiled extract probably resulted from non-enzymatic acetylation. No activity was observed if the sulfhydryl compounds were omitted from the reaction mixture, or if Triton X100 was used as control instead of boiled extract (results not presented). The latter control was used since the Triton X100 that was present in the extracts seemed to be affected by boiling. It is noteworthy that β-mercaptoethanol is acetylated more readily than L-cysteine. However, cysteine does not form stable thiol esters[4] and this might account for the relatively low activity observed with this compound. Extracts prepared with the same buffer except for the Triton X100 had no activity above the level of boiled controls, indicating that the enzyme or enzymes involved are probably membrane-bound.

The above data present evidence that extracts of mung bean roots enzymatically acetylate sulfhydryl compounds in the presence of acetyl–CoA. At present we do not know whether one or several enzymes are responsible for the acetylation or what is the physiological role of this activity. Brady and Stadtman[1] reported the fractionation of three different thiol acetyltransferases from pigeon liver extract: (1) hydrogen-sulfide acetyltransferase (E.C. 2.3.1.10); (2) thioethanolamine acetyltransferase (E.C. 2.3.1.11); (3) lipoate acetyltransferase (E.C. 2.3.1.12). Of the above enzymes lipoate acetyltransferase has mostly been investigated and in its physiological role, the enzyme catalyzes a transfer of the acetyl group from the *S*-acetyldihydrolipoyl moiety of the pyruvate dehydrogenase complex to CoA.

EXPERIMENTAL

Enzyme preparation. Roots from 11–12-day-old light grown mung bean (*Phaseolus aureus*) seedlings were extracted with acetone previously chilled to −12°. The plant residue was collected by filtration on a Buchner funnel and dried under vacuum. The dried powder was stored at −12° until used. The acetone powder was extracted with 0·05 M-K-phosphate buffer, pH 7·0, containing 1 mM-EDTA and 1% Triton X100 by stirring for 1 hr at 4°. The acetone powder to extracting buffer ratio was 1:80 (w/v). The suspension was filtered through cheese cloth, centrifuged at 20 000 *g* for 15 min and the supernatant was used for the acetylation assays.

Acetylation assay. The reaction mixture contained 0·2 ml extract, 0·1 μCi [1-^{14}C]acetyl–CoA, specific activity 48·6 mCi/mmol (New England Nuclear) and 5 mM of SH compound in a final volume of 0·4 ml in 0·1 M K phosphate buffer at pH 7·0. Incubation was carried out for 1 hr at 25°. Controls were made by heating the extract at 100° for 10 min, or by omitting the SH compound from the reaction mixture. At the end of the incubation period the reaction mixtures were applied to small columns (0·5 × 5·0 cm) containing Dowex 1 × 8 (Cl^- form, 200–400 mesh). Each column was washed with 1·4 ml H_2O and the effluents were collected directly into vials and counted in 10 ml aquasol (New England Nuclear) in a Packard Tricarb liquid scintillation spectrometer. This method can be used with positively charged or neutral SH compounds. In all cases, each experiment was done at least twice.

[4] E. R. STADTMAN, *J. Biol. Chem.* **196**, 535 (1952).